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THE GENETIC VARIATIONS IN MITOCHONDRIAL D-LOOP SEQUENCE FOR LOCAL DUCKS IN IRAQ

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Abstract

This study aimed to detect the variance in the displacement loop (D-loop) gene region and to identify some of the molecular characteristics in this area. A 718 bp region was selected from the D-loop area. Twenty-eight of local white ducks samples were used and designed accordingly the Forward primer 5'- GTTGCGGGTTATTTGGTTA -3 'and Reverse primer 5'- CCATATACGCCAACCGTCTC -3'. The results of PCR polymerization and electrolysis of the product demonstrated that the success of the amplification process and the particle size was 718bp. After analyzing the sequence of the nitrogen bases of the studied piece, observed a differences in seven individual haplotypes of nitrogen bases to the D-loop area. The value of haplotype was 0.667, and four of them were independent and 3 were shared with ducks from different countries. From the results, local Iraqi ducks were closer in terms of the tree of evolution and genetic distance between them was lower than in any other country, which maybe means that the origin of local ducks is Chinese ducks. The rate of molecular variation of AMOVA for the D-loop region between breeds was higher than that of breeds within breeds. The pieces obtained in this study for D-loop were recorded in the NCBI, EMBL and DDBJ genealogies and under independent accession numbers for our local LC480437, LC480438, LC480439, LC480440 and LC480441.

Keywords: D-loop; Duck; Genetic; mitochondrial DNA

Introduction

Ducks belong to several families such as *Anatidae, Anseriformes* and *Anas platyrhynchos* (Cheng,1995) . The main purpose of duck rising in Asia is to produce meat and eggs. Recently, the modern technology of molecular genetics has introduced a number of genetic markers that have helped researchers in genetic analysis and genetic diversity assessment, differentiate types and species of breeds to maintain them as sources of diversity. This can be relied upon as one of the sources of natural wealth in most countries. The markers have contributed to provide information at the molecular level to different regions of the genome (Sharma *et al.*, 2015). The variation in the mitochondrial DNA (mtDNA) is one of the most important sources of genetic variation. The role of mitochondrial DNA is identical that one is found in nuclear DNA of nucleus, where it can form different types of RNA which are rRNA, tRNA and mRNA. Moreover it can also translate mRNA into proteins within mitochondria (Bensasson *et al.*, 2000). The mitochondrial DNA is a short acid of approximately 16,000 base pairs (Boore, 1999). Mitochondria have been used in several fields and have played a major role in the detection of traits in humans and animals and in many modern scientific applications (Matsuda *et al.*, 2005; Nelson and Melton, 2007). Due to the lack of information on mitochondrial genes on local ducks has made. It is necessary to provide information on biodiversity and genetic variation, knowledge of strain expansion and knowledge of mtDNA mutations. The study aims to provide some of this information to the Dloop region in mtDNA. Where is the aim(s) of this study, you need to write the aim(s) here.

Methods and Materials

In this study, 28 domestic Iraqi white ducks were collected to take blood samples from their wings by taking 5 ml of blood and a small syringe were used for that to each bird and injected in 5 ml test tubes containing EDTA. The process of extracting DNA was performed using the kits test. These kits were supplied by the Korean company Genaid. The verification process was performed for DNA extraction process using electrophoresis with 1% agarose gel, and the Ethidiume Bromide dye. The process included addition the DNA marker to 100 base pairs. The DNA purity and its concentration were estimated by using Nanod device rop 2000. The DNA concentration ranged between 25.4-63.6 ng/µl and the purity (260/280) ranged between 1.67-1.99. These ranges were selected based on the recommended ranges for successful amplification (Dauphin *et al.*, 2011).

A 718 bp segment was selected from the D-loop region of mtDNA. The beginning primer 5'-GTTGCGGGTTATTTGGTTA- and reverse primer 5'-CCATATACGCCAACCGTCTC-3' were selected based on Purwantini *et al.* (2013). The fragment recorded under HM010684. 1, which includes the entire mitochondrial genome mtDNA. The following Table (1) shows the program for the selected product from the DLoop region.

Table 1 : The amplification program for the selected product from the DLoop region.

No. Cycles	Time (min)	Temperature	Stages
1	4	95C	Initial Denaturation
31	0.30	95C	Denaturation
	0.30	60C	Annealing
	0.35	72C	Extension
1	10	72C	Final Extension

After completion of the amplification process, the PCR product was sent to Yang ling tiantun aoka biotechnology company in China for the purpose of obtaining sequence of nitrogen bases (sequencing), the sequencing of the studied region and all the samples as shown in Figure 1.

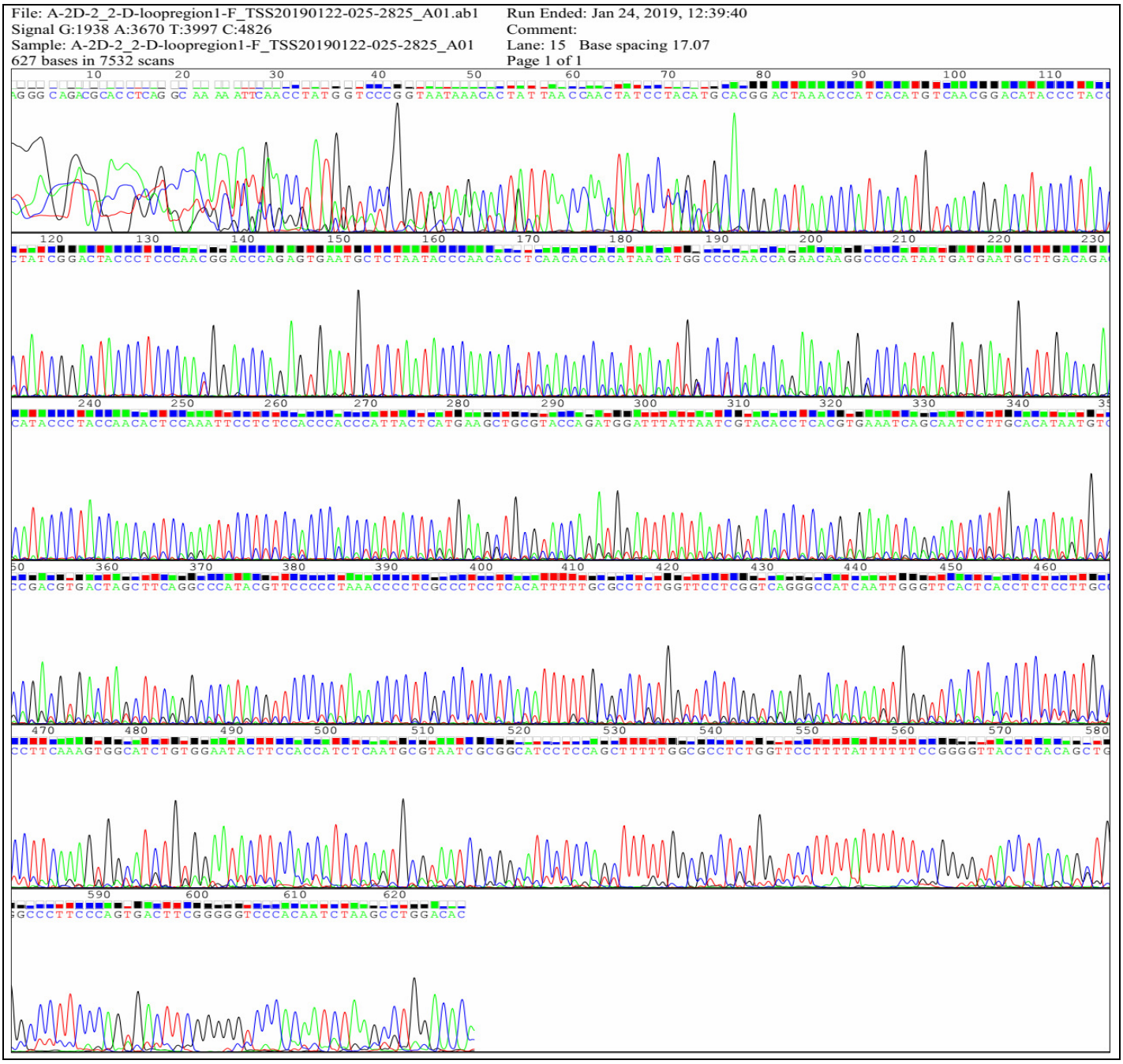

Fig. 1 : The sequencing of the studied DLoop region.

DNAp V 5.10 program was used to calculate the diversity of individual patterns of Haplotype diversity (HD) and the nucleotide diversity (Librado and Rozas, 2009). Network V 5.0.1.1program was used to plot the individual pattern network for the D-loop region based on the Median Joining MJ (Bandelt *et al.*, 1999). The individual pattern network of experimental birds was compared with the individual patterns of breeds from different countries to plot the phylogenetic tree using Mega 7 V.0.26 program in the neighbor-linking tree method (NJT) to illustrate relationships between individual or clan patterns. Arlequin ver. 3.5.1.2 program was used to calculate the genetic distance and the molecular variance analysis (AMOVA) and the neutrality test, which includes the Tajima statistic, D and Fs (Excoffier & Lischer, 2010).

Results & Discussion

The results showed that the amplification process of the PCR technique used in the experiment was successful. The product gave the size of 718 base pairs as shown in Figure 2.

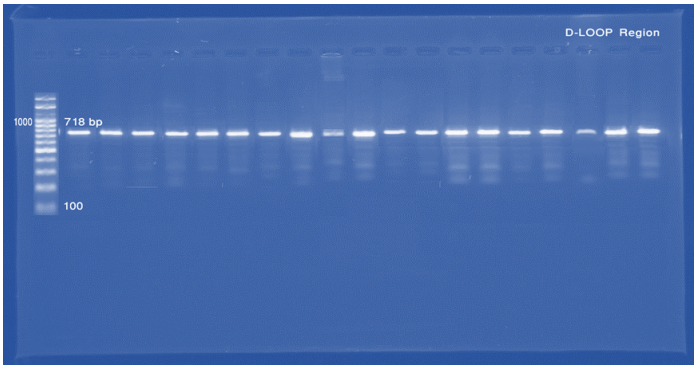

Fig. 2 : Agarose Gel Electrophoresis for the gene Dloop region of 718 base pairs.

The results of Table 2 showed some molecular measurements of the D-loop region in local Iraqi ducks that illustrated presence of 7 changes in different sites of nitrogen bases, which produced seven individual haplotypes, and the value of the variance of individual patterns is Haplotype 0.667. Meanwhile, the value of the Nucleotide diversity for the studied region was 0.00388. This refers that the percentage of change in the nitrogen bases of the Dloop region in local Iraqi ducks is low.

Table 2 : Some Molecular Measurements of the Dloop region of the Local Iraqi ducks

	Molecular Measurements		the value
1.	Haplotypes	Product Size	718
		Mutations	7
		No. of Haplotypes	7
		(HD) Haplotype diversity	0.667
		Nucleotide diversity (π)	0.00388
2.	Neutrality Test	*Tajima's D Statistic*	0.70723
		Fu's F Statistic	1.27760
3.	Mismatch distribution	Average Number of Pairwise Differences	2.222
		Raggedness statistic r	0.0652

For Neutrality Test, Tajima's D Statistic was 0.7072 and this value is close to zero, which infer that the clan is more stable and not under evolutionary pressures (Tajima, 1989). Fu's F statistic test recorded a positive values 1.27760, which means that observing the lowest positive possible number of individual patterns, mean to get lower number of individual patterns in clan (Fu, 1997), which it is clear in evident in the low rate of excretion of the gene in this study. While Mismatch distribution indicated the values of Pairwise Differences and Raggedness statistic r, and It appears that the population is stable and in demographic equilibrium (Jobling *et al.*, 2004). Gaur *et al.* (2017) found For his study of ducks in Australia, the value of Dloop individual variation was 0.8235 and the value of nucleotide diversity was 0.52.

The total number of individual haplotypes was 23, as shown in Figure 3. The individual patterns of the current study animals were recorded in four patterns, which were namely H2, H5 H7 and H8. The remaining three were shared with other countries. On the other hand, H6 was between local ducks and Dutch ducks. H4 shared the style of the local duck with Chinese ducks and American ducks.

The individual patterns of network included three individual groups: haplogroupes. The first group consisted of Indonesian ducks, with individual patterns belonging to China and the Netherlands. The second group belonged to the local ducks and the Dutch ducks. The third group of patterns consisted mainly of local ducks, Chinese and American.

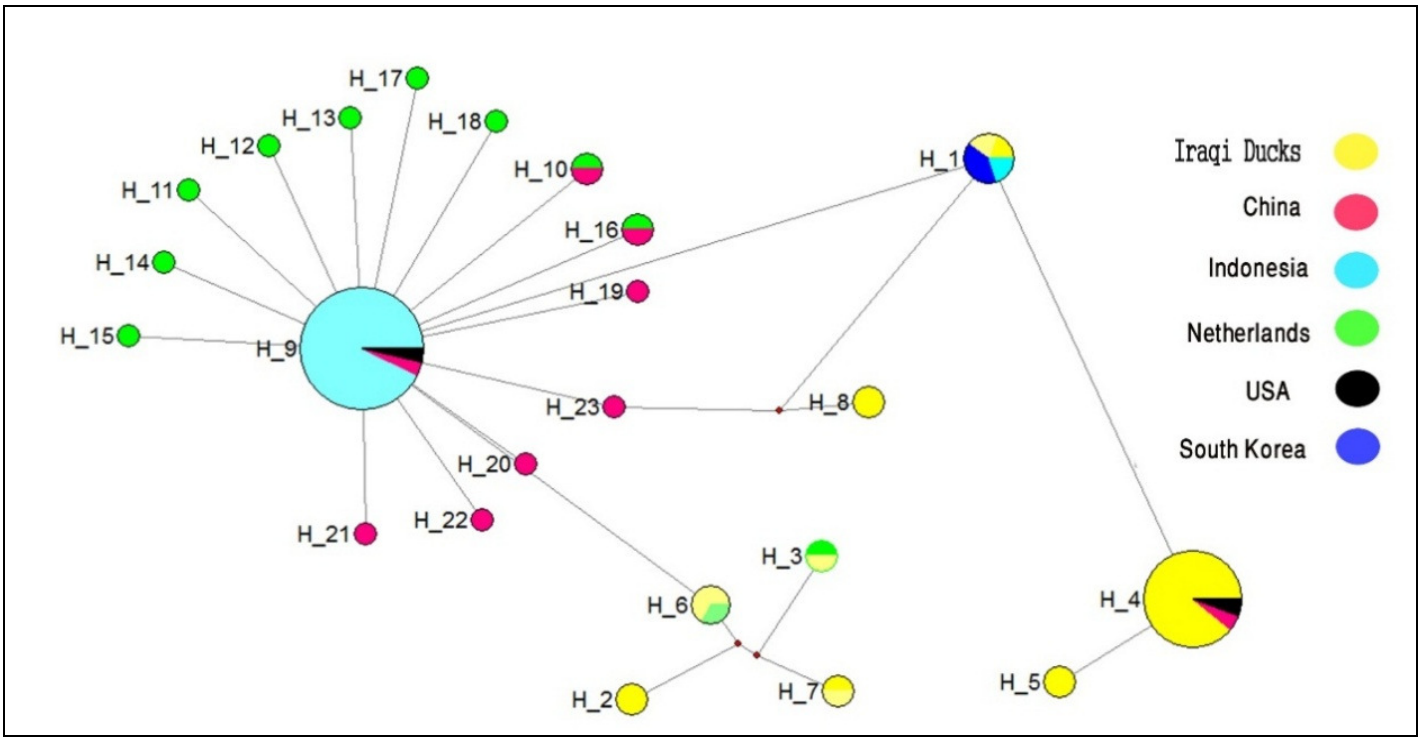

Fig. 3 : Distribution of individual patterns of Dloop region in local Iraqi ducks as well as breeds from different countries.

Since neighbor-joining tree (NJT) trees are used to illustrate the relationships between individual patterns in animals, these trees (NJT) have been used to illustrate the relationship between studied animals and global breeds. Fig. 4 showed the results of the tree that has three main branches of the tree adjacent to the Dloop region of the duck. The Russian ducks occupied a separate branch and the second branch of the American and the Andean ducks. The third main branch was divided into two main branches of the Dutch duck and the other of the local duck, Chinese and Korean. Evolution and development of ducks Is the closest to Chinese and Asian ducks in general, and this proves the view that the origin of the local ducks is the Chinese ducks.

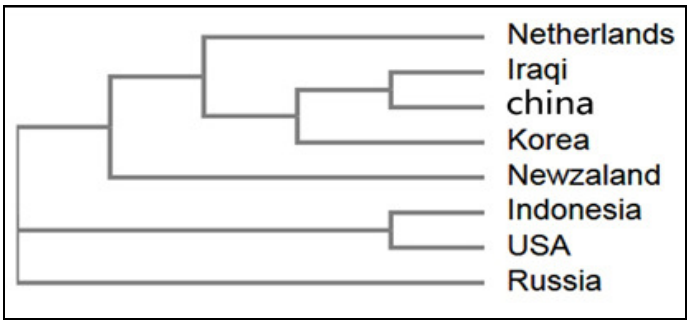

Fig. 4 : The Neighbor-joining tree of the Dloop region of the local ducks compared to the International breeds.

The results the analysis of molecular variance (AMOVA) is shown in Table 3. This table illustrated that the variance among the breeds is high with a total of 391.728 with a percentage of 72.48% compared to variation within the breeds. The total number of squares was 179.596 and has 27.52%. This might be referred to that the tribes are balanced and maintain with minor changes of their breeds. The results differed with the results that reported by Paramasivam *et al.* (2013) who studied the Indonesian ducks. They indicated that the genetic variance among breeds was higher compared to the genetic variation within the breeds, where it reached 24.88 and 75.12%, respectively. Gaur *et al.* (2017) also pointed out that the genetic variation within the breeds is higher than the genetic variation between the breeds.

Table 3 : Analysis of molecular variation AMOVA for Dloop region of local ducks and ducks from different countries.

Percentage of Variation	Variance Components	Sum of squares	d.f.	
72.48	7.38957	391.728	6	Among populations
27.52	2.80619	179.596	64	Within populations
	10.19576	571.324	70	Total
Fixation Index FST : 0.72477				

The genetic distances between the local and global breeds were expressed using the Fst index. Results of the analysis using Mega 7 software showed the highest genetic distance between the local ducks and the Indonesian ducks was 0.004554. The lowest genetic distance between local ducks and Chinese ducks was 0.001452, as shown in Table 4. This study showed that all genetic distances among breeds was low, and these results consistence with the results that reported Harti and Clark (1997).

Table 4: The genetic distance of the MC1R gene for local ducks and ducks from different countries.

	Iraqi Ducks	Indonesia	Korea South	Newzealand	China	USA	Russia
China	0.001452						
Russia	0.001732	0.000000					
Newzealand	0.003509	0.001748	0.001748				
Korea South	0.003509	0.001748	0.001748	0.000000			
USA	0.003509	0.001748	0.001748	0.000000	0.000000		
Indonesia	0.004554	0.001748	0.001748	0.000000	0.000000	0.000000	

The parts were obtained in this study are recorded in the Dloop region of the Global Genebank Sites (Annex 1) in NCBI, EMBL and DDBJ under independent accession numbers for our local Iraqi population as detailed in Table 5.

Table 5: Accession Numbers for Dloop region oflocal ducks for our study

No.	Accession number	Name	Fragment Size
1-	LC480437	Dloop region	584 bp
2-	LC480438	Dloop region	584 bp
3-	LC480439	Dloop region	584 bp
4-	LC480440	Dloop region	584 bp
5-	LC480441	Dloop region	584 bp

Conclusions

The results of this study showed that the genetic variance of the Dloop was higher than that one found in other breeds. The local Iraqi ducks may be originated from Chinese ducks based on the results of the network of individual patterns, and the genetic distances and the tree of evolution.

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